

What Is Claimed Is:

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1. A non-pyrogenic, endotoxin-free, oxygen-free, stroma-free, cross-linked tetrameric hemoglobin.
 2. The hemoglobin of claim 1, wherein said hemoglobin has been cross-linked with bis dibromo salicyl fumarate.
 3. The hemoglobin of claim 1, wherein said hemoglobin has been modified by reaction with pyridoxal-5'-phosphate.
 4. The hemoglobin of claim 2, wherein said hemoglobin has been modified by reaction with pyridoxal-5'-phosphate.
 - 10 5. The hemoglobin of claim 1, wherein said hemoglobin is human hemoglobin.
 6. The hemoglobin of claim 1, wherein said hemoglobin is bovine or porcine hemoglobin.
 - 15 7. A blood substitute composition comprising a preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin, and a pharmaceutically acceptable carrier.
 8. The blood substitute of claim 7, wherein said hemoglobin has been cross-linked with bis dibromo salicyl fumarate.
 - 20 9. The blood substitute of claim 7, wherein said hemoglobin has been modified by reaction with pyridoxal-5'-phosphate.
 10. The blood substitute of claim 8, wherein said hemoglobin has been modified by reaction with pyridoxal-5'-phosphate.
 11. The blood substitute of claim 7, wherein said hemoglobin is human hemoglobin.

12. The blood substitute of claim 7, wherein said hemoglobin is bovine or porcine hemoglobin.
13. A method of supplementing the blood of a mammal which comprises administering to said mammal a blood substitute composition comprising a preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin and a pharmaceutically acceptable carrier.
14. The method of claim 13, wherein said hemoglobin is cross-linked with bis dibromo salicyl fumarate.
15. The method of claim 13, wherein said hemoglobin is modified by reaction with pyridoxal-5'-phosphate.
16. The method of claim 14, wherein said hemoglobin is modified by reaction with pyridoxal-5'-phosphate.
17. The method of claim 13, wherein said hemoglobin is human hemoglobin.
18. The method of claim 13, wherein said hemoglobin is bovine or porcine hemoglobin.
19. A preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin produced by the process comprising the steps of:
 - (A) removing endotoxin from a preparation containing red blood cells;
 - (B) removing oxygen from said preparation containing red blood cells; and
 - (C) lysing red blood cells.
20. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 19, wherein in step (B), said

oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation.

21. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 20, wherein said process additionally comprises the steps of:

- (D) separating hemoglobin from the stroma of said lysed red blood cells; and
(E) cross-linking said separated hemoglobin.

22. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 21, wherein said process step (A) additionally comprises washing surfaces and equipment that will come into contact with the cross-linked hemoglobin with a dilute solution of a hemoglobin.

23. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 20, wherein said process step (B) comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.

24. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 21, wherein said red blood cells are human red blood cells.

25. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 21, wherein said red blood cells are bovine or porcine red blood cells.

26. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 21, wherein said preparation additionally contains a pharmaceutically acceptable carrier.

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27. A preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin produced by the process comprising the steps of:

- (A) removing endotoxin from a preparation containing red blood cells;
- (B) lysing red blood cells; and
- (C) removing oxygen from hemoglobin of said lysed red blood cells.

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28. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 27, wherein in step (C), said oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation.

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29. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 27, wherein said process additionally comprises the steps of:

- (D) separating hemoglobin from the stroma of said lysed red blood cells; and
- (E) cross-linking said separated hemoglobin.

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30. The preparation of non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 29, wherein said process step (A) additionally comprises washing surfaces and equipment that will come into contact with the cross-linked hemoglobin with a dilute solution of a hemoglobin.

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31. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 28, wherein said process step (C) comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.

32. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 29, wherein said red blood cells are human red blood cells.

33. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 29, wherein said red blood cells are bovine or porcine red blood cells.

34. The preparation of endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 29, wherein said preparation additionally contains a pharmaceutically acceptable carrier.

35. A method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin comprising the steps of:

(A) removing endotoxin from a preparation containing red blood cells;

(B) removing oxygen from said preparation containing red blood cells; and

(C) lysing red blood cells.

36. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 35, wherein in step (B), said oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation..

37. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 36, wherein said method additionally comprises the steps of:

(D) separating hemoglobin from the stroma of said lysed red blood cells; and

(E) cross-linking said separated hemoglobin.

38. The method of claim 35, wherein said process step (A) additionally comprises washing surfaces and equipment that will come into contact with the cross-linked tetrameric hemoglobin with a dilute solution of a hemoglobin.

5 39. The method of claim 36, wherein said process step (B) additionally comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.

40. The method of claim 37, wherein said hemoglobin is human hemoglobin.

10 41. The method of claim 37, wherein said hemoglobin is bovine or porcine hemoglobin.

42. A method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin comprising the steps of:

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- (A) removing endotoxin from a preparation containing red blood cells;
 - (B) lysing red blood cells; and
 - (C) removing oxygen from hemoglobin of said lysed red blood cells.

43. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 42, wherein in step (C), said oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation..

20 44. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 43, wherein said method additionally comprises the steps of:

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- (D) separating hemoglobin from the stroma of said lysed red blood cells; and
 - (E) cross-linking said separated hemoglobin.

45. The method of claim 42, wherein said process step (A) comprises washing surfaces and equipment that will come into contact with the cross-linked tetrameric hemoglobin with a dilute solution of a hemoglobin.
- 5 46. The method of claim 43, wherein said process step (C) additionally comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.
47. The method of claim 44, wherein said hemoglobin is human hemoglobin.
- 10 48. The method of claim 44, wherein said hemoglobin is bovine or porcine hemoglobin.
49. A method of increasing the oxygen carrying capacity of an individual which comprises administering to said individual a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin.
- 15 50. The method of claim 49 for increasing an individual's oxygen carrying capacity, wherein said non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin is administered by transfusion or injection.
51. The method of claim 49 for increasing an individual's oxygen carrying capacity, wherein said non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin is produced by a process comprising the steps:
 - 20 (A) removing endotoxin from a preparation containing red blood cells;
 - (B) removing oxygen from said preparation containing red blood cells; and
 - 25 (C) lysing red blood cells.

52. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 51, wherein in step (B), said oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation.
- 5 53. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 51, wherein said process additionally comprises the steps of:
 - (D) separating hemoglobin from the stroma of said lysed red blood cells; and
 - 10 (E) cross-linking said separated hemoglobin.
54. The method of claim 51, wherein said process step (A) additionally comprises washing surfaces and equipment that will come into contact with the cross-linked tetrameric hemoglobin with a dilute solution of hemoglobin.
- 15 55. The method of claim 52, wherein said process step (B) additionally comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.
56. The method of claim 53, wherein said hemoglobin is human hemoglobin.
- 20 57. The method of claim 53, wherein said hemoglobin is bovine or porcine hemoglobin.
58. The method of claim 49 for increasing an individual's oxygen carrying capacity, wherein said non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin is produced by a process comprising the steps:
 - 25 (A) removing endotoxin from a preparation containing red blood cells;

- (B) lysing red blood cells; and
- (C) removing oxygen from hemoglobin of said lysed red blood cells.

59. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 58, wherein in step (C), said oxygen is removed by centrifuging the red blood cells under a vacuum sufficient to remove oxygen from the preparation.

60. The method for producing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin of claim 58, wherein said process additionally comprises the steps of:

- (D) separating hemoglobin from the stroma of said lysed red blood cells; and
- (E) cross-linking said separated hemoglobin.

61. The method of claim 56, wherein said process step (A) comprises washing surfaces and equipment that will come into contact with the cross-linked tetrameric hemoglobin with a dilute solution of hemoglobin.

62. The method of claim 59, wherein said process step (C) additionally comprises centrifuging a solution of said cells under vacuum at a speed sufficient to produce a force greater than the surface tension of the solution.

63. The method of claim 60, wherein said hemoglobin is human hemoglobin.

64. The method of claim 60, wherein said hemoglobin is bovine or porcine hemoglobin.

65. A container containing a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin composition.

66. The container of claim 65, which is an anoxic container composed of polyethylene terephthalate.

- 5 67. The container of claim 65, which is an implantable delivery device that delivers a non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin composition to a recipient.
68. The container of claim 65, wherein said non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin contained therein is produced through the process comprising the steps of:
- (A) removing endotoxin from a preparation containing red blood cells;
 - (B) removing oxygen from said preparation containing red blood cells;
 - (C) lysing red blood cells;
 - (D) separating hemoglobin from the stroma of said lysed red blood cells; and
 - (E) cross-linking said separated hemoglobin.
- 10 69. The container of claim 65, wherein said non-pyrogenic, endotoxin-free, stroma-free, cross-linked tetrameric hemoglobin contained therein is produced through the process comprising the steps of:
- (A) removing endotoxin from a preparation containing red blood cells;
 - (B) lysing red blood cells;
 - (C) removing oxygen from hemoglobin of said lysed red blood cells;
 - (D) separating hemoglobin from the stroma of said lysed red blood cells; and
 - (E) cross-linking said separated hemoglobin.
- 15 70. The container of claim 65, wherein said hemoglobin is human hemoglobin.
- 20 71. The container of claim 65, wherein said hemoglobin is bovine or porcine hemoglobin.
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